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Urocystis cumminsii sp. nov., a smut fungus on *Themidaceae* from Arizona

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Abstract—The morphology and phylogenetic relationships of a species of *Urocystis* on *Dichelostemma capitatum* (*Themidaceae*, *Asparagales*) collected in the Tucson Mountains in Arizona, United States, were studied using microscopy and ITS rDNA sequence analyses. This is a first record for smut fungi on hosts from *Themidaceae.* Molecular phylogenetic analyses based on ITS sequence data revealed its basal position in relation to species on *Poaceae.* As a result, the smut in leaves of *Dichelostemma capitatum* is described and illustrated here as a new species, *Urocystis cumminsii.*

Key words—plant pathogens, taxonomy *Urocystidales*

Introduction

Urocystis Rabenh. ex Fuckel contains more than 170 species of plant pathogenic smut fungi, found all over the world but more common in temperate areas of both hemispheres (Vánky 2011b). More than 60% of *Urocystis* species are found on monocotyledons, with *Poaceae* serving as a major monocotyledonous host family, followed by *Juncaceae*, *Hypoxidaceae*, *Convallariaceae*, *Amaryllidaceae*, and *Hyacinthaceae* (Vánky 2011b). The intra-level phylogenetic relationships of *Urocystis* species have never been analysed and thus far only the graminicolous species from triticoid hosts

have been included in comprehensive phylogenetic analyses (Savchenko & al. 2017). Most of the *Urocystis* species described recently—e.g., *U. achnatheri* L. Guo, *U. anemones-narcissiflorae* Vánky, *U. arxanensis* L. Guo, *U. beckwithiae* Vánky, *U. circaeasteri* Vánky, *U. dunhuangensis* S.H. He & L. Guo, *U. glabella* Vánky & R. G. Shivas, *U. helanensis* L. Guo, *U. koeleriae* L. Guo, *U. phalaridis* Vánky, *U. puccinelliae* L. Guo & H.C. Zhang, *U. rostrariae* Piątek, *U. sinensis* L. Guo, *U. skirgielloae* Piątek, *U. wangii* L. Guo, and *U. xilinhotensis* L. Guo & H.C. Zhang—were supported only by morphological and host-specialization data (Guo 2002, 2005, 2006; Guo & Zhang 2004, 2005; He & Guo 2007; Piątek 2006a, 2006b; Vánky 2004, 2005, 2011a; Vánky & Abbasi 2011). The integration of molecular phylogenetic analyses, host plant taxonomy, and morphology provides a natural classification for various smut genera (Bauer & al. 2008; Castlebury & al. 2005; Kruse & al. 2018; Lutz & al. 2008; McTaggart & al. 2012; Savchenko & al. 2013, 2015; Vánky & Lutz 2007), justifying the need of a phylogenetic study of the genus.

During a survey of *Urocystis* species diversity in the United States, we examined a specimen identified as *Urocystis* sp. on *Dichelostemma capitatum* (Benth.) Alph. Wood (*Themidaceae*) from Arizona in the WSP herbarium. Previously, no *Urocystis* species had been recorded on hosts from this family. The present study aimed to resolve the specific status of the smut on *D. capitatum* through morphological analysis and determine its phylogenetic affinities within *Urocystis*.

Table 1. GenBank sequences used in this study.

Species	GenBank no.	Reference
Antherospora scillae	EF653983	Bauer & al. 2008
A. vaillantii	EF653988	Bauer & al. 2008
Urocystis bolivarii	KX057771	Savchenko & al. 2017
U. colchici	DQ839596	Matheny & al. 2006
U. cumminsii	MK575496	This study
U. eranthidis	JN367299	Kellner & al. 2011
U. fischeri	KF668284	Smith & Lutz 2013
U. occulta	KX057774,	Savchenko & al. 2017
U. trillii	HQ239361	Henricot 2010
U. tritici	KX057782	Savchenko & al. 2017
Ustilago hordei	AY345003	Stoll & al. 2003
Vankya heufleri	EF667965	Bauer & al. 2008
V. ornithogali	EF635910	Bauer & al. 2008

Materials & methods

The herbarium specimen is deposited in Washington State University Mycological Herbarium, Pullman, WA, United States (WSP).

Sorus and spore characteristics were studied using dried herbarium material. Specimens were examined by light microscopy (LM). Pictures of sori were taken with a Canon Power Shot G10 camera. For LM, spores were mounted in 90% lactic acid on a microscope slide, gently heated to boiling point to eliminate air bubbles, and then examined under a Carl Zeiss Axiostar™ light microscope at 1000× magnification and photographed with a Canon Power Shot G10 camera. At least 50 spore balls were measured, and the variation is presented as a range with extreme values given in parentheses. Means and standard deviations (SD) are provided after the spore size ranges.

For SEM studies, spore balls were attached to metal stubs by double-sided adhesive tape and coated with gold. Spore surface ornamentation was observed at 15 kV and photographed with a JEOL JSM-6700F scanning electron microscope with a working distance of c. 12–13 mm.

Sequences from other species of *Urocystis* and related genera were obtained from GenBank (Table 1). Genomic DNA was isolated from spore balls removed from the herbarium specimen that had been lysed in 1.5 mL tubes for 1 min using FastPrep®24. Tubes were incubated in a water bath for 5 hours at 55 °C, and DNA extracted using DNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions.

DNA was amplified in 20 µl aliquots on an Applied Biosystems® GeneAmp 9700 thermal cycler using ITS1 as the forward primer and ITS4 as the reverse primer (White & al. 1990).

Standard cycling parameters with an annealing temperature of 57 °C were used for amplification. PCR products were purified with USB ExoSAP-IT according to the manufacturer's instructions, amplified with the forward and reverse PCR primers with the BigDye® Terminator v3.1 Cycle Sequencing Kit, and sequenced on an ABI PRISM® 3100 Genetic Analyzer.

Consensus sequences were assembled, aligned, and edited with Geneious 7.1.8 for MacOS and with MAFFT 6.853 (Katoh & al. 2002, Katoh & Toh 2008) using the L-INS-i option. Maximum Likelihood (ML) was implemented as a search criterion in RAxML (Stamatakis 2014). GTR+I+G was specified as the evolution model in MrModeltest (Nylander & al. 2004). The RAxML analyses were run with a rapid Bootstrap analysis (command –f a) using a random starting tree and 1000 maximum likelihood bootstrap replicates. A Markov Chain Monte Carlo (MCMC) search in a Bayesian analysis (BA) was conducted with MrBayes (Ronquist & Huelsenbeck 2003). Four runs were implemented for 5 million generations. The cold chain was heated to a temperature of 0.25°C. Substitution model parameters were sampled every 500 generations and trees were saved every 1000 generations. Convergence of the Bayesian analysis was confirmed using AWTY (Nylander & al. 2008) and a

burn-in of 18,000 generations was calculated. The ML and Bayesian analyses were run three times to test accuracy. The tree was rooted using *Ustilago hordei* (Pers.) Lagerh.

Results

The ITS alignment of 13 sequences (including the outgroup *Ustilago hordei*) comprised 643 characters including gaps. The different BA and ML analytical runs yielded consistent topologies in respect to well-supported branches (a posteriori probability >90% in most cases). The consensus tree of one run of Bayesian phylogenetic analyses is presented in Fig. 1. The *Urocystis* sequences fell into two major clades. The first clade comprised species from *Poaceae* and *Themidaceae*, and the second clade included species from *Cyperaceae*, *Liliaceae* s.l., and *Ranunculaceae*. The two species from the genus *Vankya* Ershad clustered together with the second *Urocystis* clade.

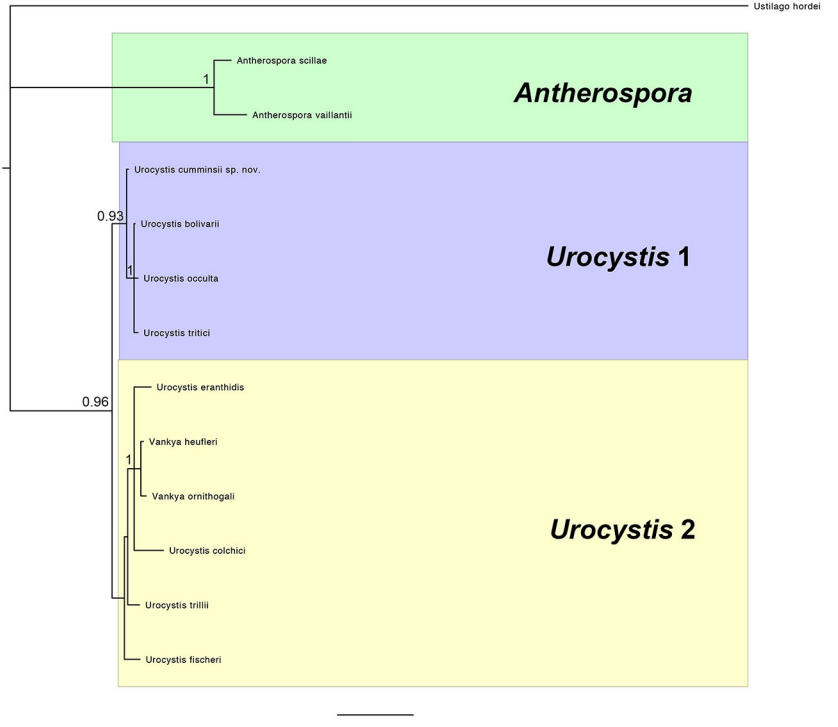

Fig. 1. Bayesian inference of phylogenetic relationships resulting from the analysis of ITS nucleotide sequence data. Numbers on branches are estimates for PPs from Bayesian inference (only probabilities >0.8 are shown).

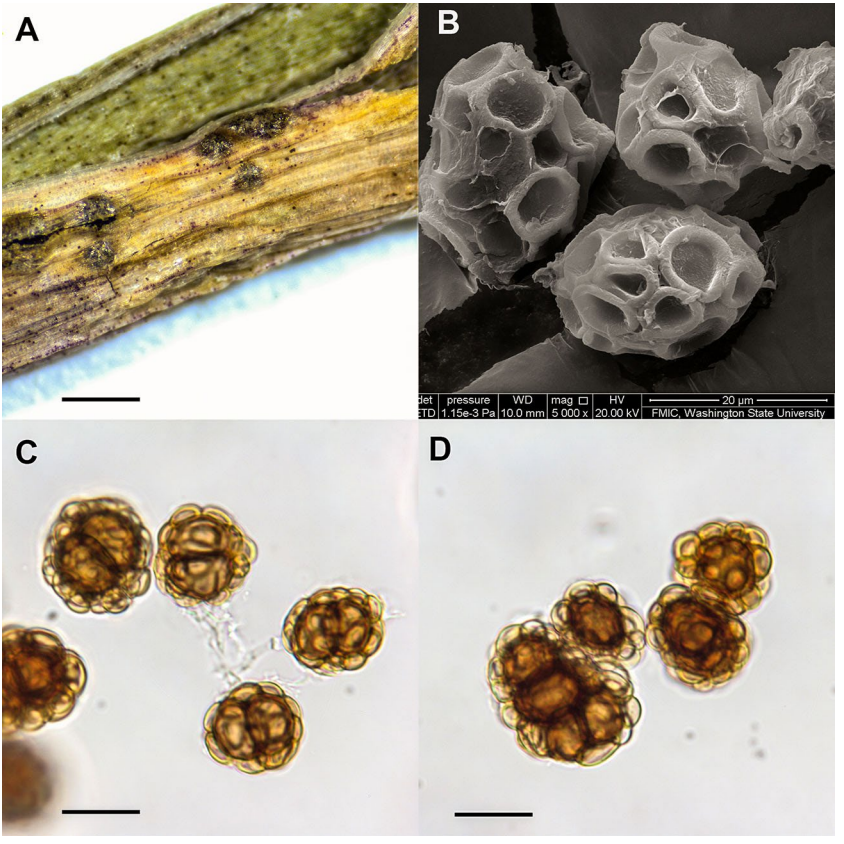

Fig. 2. *Urocystis cumminsii* (WSP 68198). A. sori in leaves of *Dichelostemma capitatum*; B. spore balls seen by SEM; C, D. spore balls seen by LM. Scale bars: A = 2 mm, B = 5 µm, C, D = 20 µm.

Taxonomy

Urocystis cumminsii K.G. Savchenko, Carris & Castl., **sp. nov.** Fig. 2

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Differs from *Urocystis camassiae* by its lighter colored yellowish-brown spores with thicker walls and its smaller thinner walled sterile cells, and by its host specialization on *Themidaceae*.

Type: USA. Arizona, King's Canyon, 16 km west of Tucson, on *Dichelostemma capitatum* (as *D. pulchellum*), 30.03.1981, leg. G.B. Cummins (**Holotype,** WSP 68198; GenBank MK575496).

Etymology: Named after George B. Cummins (1904–2007), an eminent American mycologist, who collected the holotype specimen.

Sori in leaves as slightly elevated, pustular, elongate areas of various size and shape, sometimes confluent, visible on both sides of the leaf, initially lead-colored and covered by the epidermis which ruptures exposing the powdery, black mass of spore balls. Spore balls globose, subglobose, ovoid to irregular, 20–45 µm diam., composed of 1–6 (mostly 3) spores and more or less complete investing layer of sterile cells. Spores subglobose, ovoid, irregularly oblong to elongated, 11–15 × 12–18 µm diam. [mean ± SD, 13.3 ± 2.6 × 15 ± 2.9 µm], medium yellowish brown, wall 1–1.5 µm thick, smooth. Sterile cells subglobose, elongated, ovoid, 5–7 × 5–11(–13) µm, pale yellow, to almost hyaline, with smooth, 1 µm thick wall.

Discussion

Dichelostemma Kunth is a North American genus of wild hyacinths, closely related to *Brodiaea* Sm., from the family *Themidaceae*. Plants from this family are native to Central America and western North America, from British Columbia to Guatemala (Pires & Sytsma 2002, Stevens 2018). No members of *Themidaceae* were previously known to be parasitized by smut fungi (Vánky 2011b). Our molecular phylogenetic analyses and morphological data have helped resolve the systematic position of *Urocystis* on *D. capitatum*.

The only possible close relative to *U. cumminsii* might be another native North American species, *U. camassiae* Vánky, found on *Camassia* Lindl. (*Agavaceae*; Fay & Chase 1996, Pires & Sytsma 2002). However, *U. camassiae* is distinguished by its darker colored reddish-brown spores with thinner spore walls (0.5–1 µm) and its larger (5–17 µm) sterile cells with thicker walls (1–2 (–3) µm; Vánky 1994).

ITS phylogenetic analysis infers that *U. cumminsii* is sister to the clade of *Urocystis* species on grasses and separate from species found on hosts from families more closely related to *Themidaceae*, such as *U. colchici* (Schltdl.) Rabenh. ex A.A. Fisch. Waldh. and *U. trillii* H.S. Jacks., indicating multiple inter-family host jumps during the evolution of *Urocystis* species, similar to those in the closely related genus *Thecaphora* Fingerh. (Vasighzadeh & al. 2014). Interestingly, *Vankya heufleri* (Fuckel) Ershad and *V. ornithogali* (J.C. Schmidt & Kunze) Ershad also clustered within the *Urocystis* lineage (Fig. 1).

Most sequences of *Urocystis* in GenBank are derived from the LSU region. Unfortunately, our preliminary analysis showed that LSU is not informative for *Urocystis* phylogenetics. Hence, we based our current phylogeny on ITS data, utilizing the limited number of *Urocystis* ITS sequences available in GenBank. Additional sequencing is needed to resolve the taxonomy and evolutionary

relationships within *Urocystis,* as well as the phylogenetic affiliation of closely related genera, such as *Vankya*.

Urocystis cumminsii expands the occurrence of *Urocystis* species to the host family *Themidaceae*. Future combined molecular phylogenetic and morphological analyses may reveal higher diversity among *Urocystis* species in North America.

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